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American Chemistry Council
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U.S. Environmental Protection Agency
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Attention: TSCA Section 8(e) Coordinator

RE: Reproductive Toxicity Range-Finding Study of Ethylbenzene in Rats

Dear Sir or Madam:

On November 27, 2002, the American Chemistry Council Ethylbenzene Panel (Panel)[1] submitted a letter on behalf of its members pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA) to inform EPA of data from a dose range-finding study being conducted on ethylbenzene. The Panel now has some additional information from the same study and is submitting it as a supplement to the November 27, 2002 submission. The Panel has not made a determination as to whether a significant risk of injury to health or the environment is presented by these supplemental findings.

As noted in the Panel's November 27 submission, the Panel is conducting a range-finding study at WIL Research Laboratories to develop information to aid in selection of exposure concentrations for a planned 2-generation reproductive toxicity study. These studies are being conducted under the Voluntary Children's Chemical Evaluation Program (VCCEP). The range-finding study design consists of four groups of twenty male and female Sprague-Dawley rats administered ethylbenzene by whole body vapor inhalation at concentrations of 0, 100, 500 or 1,000 ppm. The inhalation exposures were given for six hours each day, seven days per week to males for a minimum of four weeks and to females for two weeks premating, during mating and through gestation day 20. No dams were exposed via inhalation from gestation day 21 through and including lactation day 4. During lactation, half of the dams (subgroup "inhalation phase") received inhalation exposures on lactation days 5 to 27. The other half of the dams (subgroup "gavage phase") received oral gavage doses of ethylbenzene or corn oil vehicle on lactation days 1 to 4 inclusive and inhalation exposures on lactation days 5 to 27. The gavage dose levels for the dams receiving 100, 500 and 1,000 ppm

[1]The members of the Ethylbenzene Panel are ATOFINA Petrochemicals, Inc., BP Amoco Chemical Company, Chevron Phillips Chemical Company, The Dow Chemical Company, GE Plastics, Lyondell Chemical Company, Nova Chemicals Inc. and Sterling Chemicals Inc.

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ethylbenzene vapor were 90, 342 and 621 mg/kg/day, respectively, divided into three equal doses and administered at approximately 2 hour intervals. The gavage dose levels and number of doses administered per day attempted to approximate the blood concentrations of the maternal inhalation exposures and were based on physiologically-based pharmacokinetic modeling. Beginning on PND 22 and continuing through PND 33, 1 pup/sex/litter from each maternal phase of the study began direct daily 6-hour inhalation exposure to ethylbenzene at the same level as their parents. Beginning on PND 29 and continuing through PND 33, an additional 1 pup/sex/litter from each maternal phase of the study began direct daily 6-hour inhalation exposure to ethylbenzene at the same level as their parents.

At the time of the November 27 submission, the lactation phase was partially complete and results indicated a statistically significant lower body weight on postnatal day 1 (PND 1) for male and female pups of dams that received 1,000 ppm ethylbenzene by inhalation (combined phases). Now the lactation and the two post-weaning direct exposure phases are completed, and some additional findings are noted. During the post-weaning exposure for the gavage phase from PND 22 - 33, 2/16 and 4/20 weanlings in the 100 ppm/90 mg/kg and 1,000 ppm/621 mg/kg groups, respectively, were found dead or euthanized *in extremis*. During the same exposure period for the inhalation phase, 1/18 and 3/17 weanlings in the 500 and 1,000 ppm groups, respectively, were found dead or euthanized *in extremis*. Clinical signs noted in both phases one hour after exposure at the 1,000 ppm level included labored respiration, eyelids half closed, prostrate, and marked difficulties in righting and ambulation. These findings were generally noted in the first several days of exposure only. In both inhalation and gavage phases, body weight gain was markedly reduced following exposure to 1,000 ppm ethylbenzene for the F1 male and female weanlings on PND 22-34. This reduction was more pronounced following the first day of exposure and among the animals in the gavage phase. In the post-weaning exposure component beginning on PND 29, mean body weight gain of pups was reduced for both the inhalation and gavage phase animals exposed to 1,000 or 500 ppm ethylbenzene following the first day of exposure.

The study is presently ongoing at the laboratory. The final report will be provided to EPA, once it becomes available.

If you have any questions, please contact me at 301 924 2006 or Elizabeth_Moran@americanchemistry.com.

Yours truly,

Elizabeth J. Moran, Ph.D.
Manager, Ethylbenzene Panel

Attachment: Interim Report - First Weaning, Second Weaning, F1 Generation Exposure and Maternal Findings, dated December 6, 2002.

Interim Report – First Weaning, Second Weaning, F_1 Generation
Exposure and Maternal Findings

A Pilot Inhalation Study for a Reproductive Toxicity Study of Ethylbenzene in Rats

WIL-186028

December 6, 2002
Donald G. Stump, Ph.D., D.A.B.T.

This draft status report encompasses the postnatal period from PND 7-28, the post-weaning exposure period for the pups weaned on both PND 21 and PND 28, and maternal findings.

Following the lactation days 1-4 gavage phase for half of the F_0 females (inhalation/gavage phase) or the period of no exposure on lactation days 1-4, (inhalation phase), all F_0 females were exposed in the control, 100, 500 or 1000 ppm chambers from lactation day 5 through 27. On postnatal day (PND) 21, one pup/sex/litter was weaned and exposed to the test article from PND 22 through 33. Weaned littermates were housed together during the exposure and non-exposure periods. The remaining pups were housed with their respective dams until weaning on PND 28. On PND 28, one pup/sex/litter was weaned and exposed to the test article from PND 29 through 33. As for the pups weaned on PND 21, the littermates were housed together during the exposure and non-exposure periods. All other pups were necropsied on PND 28

In the inhalation phase, one 500 ppm group F_0 female had a total litter loss on lactation day 0 and one female in the 100 ppm group had a total litter loss on lactation day 15; no total litter losses occurred in the inhalation/gavage phase. With the exception of the two females with total litter loss, all F_0 females that delivered survived to scheduled euthanasia on lactation day 28. Maternal clinical signs and body weight gain during lactation were unaffected by ethylbenzene exposure in both the inhalation and inhalation/gavage phases. A transient reduction in food consumption was observed during PND 7-14 in the 1000 ppm/621 mg/kg group inhalation/gavage phase females. No ethylbenzene-related necropsy findings were observed in the F_0 females. Absolute liver weights were increased relative to the control group in the 500 ppm/324 mg/kg and 1000 ppm/621 mg/kg groups by 15 and 25%, respectively, in the inhalation/gavage phase. In the inhalation phase, absolute liver weights in the 500 and 1000 ppm groups were increased by 25 and 44%, respectively. Following culling on PND 4 until PND 28 pup survival was unaffected by maternal ethylbenzene exposure in both the inhalation and inhalation/gavage phases. Mean pup body weights prior to weaning for both males and females in the 1000 ppm/621 mg/kg inhalation/gavage phase were lower (15-21% and 11-18%, respectively) than the concurrent control group values on PND 14-28. Mean male pup weights prior to weaning in the 500 and 1000 ppm groups (inhalation phase) were slightly lower than the control group (9-11% and 8-13%, respectively) on PND 14 and 21 and similar to the control group on PND 28. Mean female pup weights

prior to weaning for all exposure groups in the inhalation phase were generally similar to the control group on PND 14-28.

During the post-weaning exposure for the inhalation/gavage phase from PND 22-33, two and four weanlings in the 100 ppm/90 mg/kg and 1000 ppm/621 mg/kg groups, respectively, were found dead or euthanized *in extremis*. During the same exposure period for the inhalation phase, one and three weanlings in the 500 and 1000 ppm groups were found dead or euthanized *in extremis*. Clinical signs noted in both phases one hour following exposure at the 1000 ppm exposure level included labored respiration, eyelids half closed, prostrate, unable to right self and animal rocks, lurches or sways while ambulating. These findings were generally noted in the first several days of exposure only. In both the inhalation phase and the inhalation/gavage phase, body weight gain was markedly reduced following exposure to 1000 ppm of ethylbenzene for the F_1 male and female weanlings on PND 22-34; the reductions were most pronounced following the first day of exposure. Mean body weights in the 1000 ppm group F_1 males and females in the inhalation/gavage phase on PND 22-34 were lower than the control group by 16-25% and 18-27%, respectively. Mean body weights in the 1000 ppm group F_1 males and females in the inhalation phase on PND 22-34 were lower than the control group by 10-19% and 9-16%, respectively. Mean body weight gain for the F_1 animals in both phases exposed to 500 ppm of ethylbenzene beginning on PND 22 were also slightly reduced following the first day of exposure.

During the post-weaning exposure from PND 29-33, all animals in both phases survived to the scheduled euthanasia. No exposure-related clinical signs were observed prior to or one hour following exposure. In both the inhalation and inhalation/gavage phases, F_1 males and females exposed to 500 and 1000 ppm of ethylbenzene had reduced body weight gains on PND 29-34; the reductions were most pronounced following the first day of exposure. Mean body weights in the 1000 ppm group F_1 males and females in the inhalation/gavage phase were lower than the control group on PND 34 by 13% and 11%, respectively. Mean body weights in the 1000 ppm group F_1 males and females in the inhalation phase were lower than the control group on PND 34 by 10% and 6%, respectively.

The grand mean of the daily chamber concentrations for each group from the start of exposures were within 1% of the target concentrations with RSD values less than 2.8%. All daily mean chamber concentrations were within 10% of the target concentrations. Grand means of the daily chamber temperatures ranged from 22° to 24°C. Grand means of chamber relative humidity ranged from 45 to 52%.